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ORIGINAL ARTICLE

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Genetic deficiency of a mitochondrial aldehyde dehydrogenase increases serum lipid peroxides in community-dwelling females

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Abstract Mitochondrial aldehyde dehydrogenase 2 (ALDH2) plays a major role in acetaldehyde detoxification. The alcohol sensitivity is associated with a genetic deficiency of ALDH2. We and others have previously reported that such a deficiency influences the risk for late-onset Alzheimer's disease (LOAD), hypertension, and myocardial infarction. Then we tried to find phenotypes to which the *ALDH2* polymorphism contributes by conducting several evaluations including biochemical and functional analyses of various tissues in a community-dwelling population. Several serum proteins, lipids, and lipid peroxides (LPO) levels showed differences between the nondefective (*ALDH2*1/1*) and defective (*ALDH2*1/2* and *ALDH2*2/2*) ALDH2 individuals. However, alcohol-drinking behavior is known to affect these evaluations. Thus, we excluded the effects of alcohol-drinking behavior from the association with the ALDH2-deficient genotype through correction and found that the concentration of LPO was significantly lower in the nondefective ALDH2 females than the defective females. The effect of frequent alcohol-drinking behavior in males seems to override the phenotype of the high serum LPO level. These results indicate that the ALDH2 deficiency may enhance oxidative stress in vivo. Thus, these findings suggest that ALDH2 functions as a protector against oxidative stress and the decrease in protection may influence the onset of AD, hypertension, and myocardial infarction.

Keywords Aldehyde dehydrogenase 2 · Gene polymorphism · Lipid peroxides · Population-based study · Alzheimer's disease

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Introduction

Mitochondrial aldehyde dehydrogenase 2 (ALDH2) metabolizes acetaldehyde produced from ethanol into acetate and plays a major role in the oxidation of acetaldehyde in vivo (Bosron and Li 1986). A mutant allele, *ALDH2*2*, has a single point mutation (G → A) in exon 12 of the active *ALDH2*1* gene and is confined to Asians (Yoshida et al. 1984). The mutation results in a substitution of glutamic acid 487 to lysine (E487 K), acting in a dominant negative fashion (Crabb et al. 1989, Singh et al. 1989, Xiao et al. 1996). Individuals with the *ALDH2*2* allele exhibit the alcohol-flushing syndrome attributable to an elevated blood acetaldehyde level (Goedde et al. 1979, Crabb 1990). The *ALDH2*2* allele has been also reported to affect the metabolism of other aldehydes such as benzaldehyde, which is a metabolite of toluene (Kawamoto et al. 1994), and chloroacetaldehyde, which is generated during the metabolism of vinyl chloride (Farres et al. 1994, Yokoyama et al. 1996). In addition, ALDH2 deficiency was found to contribute to risks of hypertension (Takagi et al. 2001, Amamoto et al. 2002) and myocardial infarction (Takagi et al. 2002). However, the risks have been mainly argued through an association with alcohol consumption.

Recently, we have reported that ALDH2 deficiency is a risk factor for late-onset Alzheimer's disease (LOAD), synergistically acting with the $\epsilon 4$ allele of the apolipoprotein E gene (*APOE-$\epsilon 4$*) (Kamino et al. 2000). LOAD is a complex disease caused by multiple genetic and environmental factors: physiological, medical, nutritional,

and psychological. Oxidative stress and lipid peroxidation caused by reactive oxygen species (ROS) are reported to play an important role in the pathogenesis of neurodegenerative diseases. These diseases include Alzheimer's disease (AD) (Lovell et al. 1997, Mark et al. 1997), Parkinson's disease (Dexter et al. 1994), amyotrophic lateral sclerosis (Ferrante et al. 1997, Pedersen et al. 1998), and cerebral ischemia (for review, Chan 2001). A major source of ROS is mitochondrially derived superoxide anion radical, which gives rise to hydrogen peroxide. Hydrogen peroxide is often converted further to hydroxyl radical. Superoxide anion reacts with unsaturated fatty acids and induces membrane lipid peroxidation thereby generating reactive aldehydes, including malondialdehyde (MALD) and trans-4-hydroxy-2-nonenal (4-HNE). A strong electrophile, 4-HNE, has the ability to readily adduct cellular proteins and may damage the proteins by interacting with lysine, histidine, serine, and cysteine residues (Uchida and Stadtman 1992). Recently, we found that ALDH2-deficient transfectants exhibited increased vulnerability to treatment with 4-HNE (Ohsawa et al. 2003). The transfectants also had a decreased resistance to oxidative insult, caused by antimycin A, accompanied by an accumulation of proteins modified with 4-HNE. These findings suggest that mitochondrial ALDH2 functions as a protector against oxidative stress and its deficiency increases the damage from oxidative stress.

Geriatric diseases including LOAD are associated with many factors: genetic, lifestyle, physiological, medical, nutritional, and psychological. Thus, it is important to clarify the contributions of genetic factors and other basic background factors. In 1997, we started gene-related investigations into various geriatric diseases in the National Institute for Longevity Sciences, Longitudinal Study of Aging (NILS-LSA) (Shimokata et al. 2000). In this study, a molecular epidemiological analysis in the NILS-LSA revealed a higher concentration of lipid peroxides (LPO) in sera of ALDH2-deficient females than females carrying an active ALDH2. These results suggest that ALDH2 is involved in antioxidant defense and its deficiency enhances oxidative stress.

Subjects and methods

Molecular epidemiological study

The subjects were 2,259 participants in the NILS-LSA study. They were randomly selected community-dwelling males and females aged 40–79 years from Obu City and regions close to the NILS in Aichi Prefecture, Japan. The study protocol was approved by the Committee on the Ethics of Human Research of National Chubu Hospital and the NILS, and written informed consent was obtained from each subject. Their venous blood (7 ml) was collected into tubes containing EDTA (final 50 mM), and genomic DNA was isolated with an automated genomic DNA isolation system (model NA-1000; Kurabo, Osaka, Japan). The genotype of *ALDH2* was determined by the mismatched polymerase chain reaction (PCR)-RFLP method reported previously (Shimokata et al. 2000). In brief, 5 ng of DNA was amplified in 15 μl of PCR mixture with the primers 5'-TTACAGGGTCAACTGCTATG-3' and 5'-CCACACTCACA-3'. The amplified 131-bp DNA fragment including exon 12 of the *ALDH2* gene was digested with *Ear*I and separated by agarose gel electrophoresis. The *ALDH2*1* allele has 108 and 23 bp, and the mutant *ALDH2*2* allele 131 bp. Routine clinical evaluations included physical examination, blood pressure, blood chemistry including LDH, total cholesterol, triglyceride, HDL-cholesterol, LPO, complete blood cell count, and urine analysis. LPO was determined as thiobarbituric acid reactive substances.

Statistical analysis

Data are presented as means ± SD. LPO and other quantitative data were compared among ALDH2 genotypes by one-way analysis of variance and the Tukey-Kramer post hoc test. Alcohol drinking was defined as >5 g alcohol per day in Table 3, and alcohol consumption was assessed as a continuous (grams alcohol per day) variable in Tables 4 and 5. In Table 2, data were analyzed with an adjustment for alcohol consumption by the least squares method in a general linear model. In Table 5, data were analyzed by the general linear model with both *ALDH2* genotype and alcohol consumption used as an independent variable. A p value of 0.05 or less after correction by the number of comparisons was considered statistically significant.

Results

We first examined the distribution of the *ALDH2* genotype in the NILS-LSA study. The subjects numbered 2,259. They were community-dwelling males and females aged 40–79 years who were randomly selected from the area of the NILS. The genotype frequencies for *ALDH2*1/1*, *1/2* and *2/2* were tested in 1,137 males and 1122 females (Table 1). The overall frequencies of genotypes *1/1*, *1/2* and *2/2* were 51.1%, 40.1%, and 8.8%, respectively. There was no gender difference in the genotypic frequencies. However, the frequency of genotype *1/1* showed a trend in an increase (from 49.1% in the 40s group to 53.1% in the 70s group), depending upon age, despite no statistical significance. We tried to find a combination to show the significance. Then, only when females were divided into two groups (*ALDH2*1/1* and *1/2*) or (*2/2*) by age, the frequency of *ALDH2*2/2* was marginally, but significantly, lower in the older group (≥60 years) than the younger group (<60 years) ($p=0.03$ chi-square analysis, or $p=0.02$ by Fisher's exact test). Thus, the frequencies of these genotypes may not be constant throughout life.We examined the association of the *ALDH2*-deficient genotype with various evaluations in the NILS-LSA study. In addition to biochemical analyses of blood and urine, renal and liver functions, serum proteins and lipids, and a complete blood count, LPO and geriatric disease markers were also examined. Several serum proteins, lipids, and LPO levels showed differences between the nondefective (*ALDH2*1/1*) and the defective (*ALDH2*1/2* and *ALDH2*2/2*) ALDH2 individuals (Table 2). However, these biochemical evaluations are known to be affected by alcohol-drinking behavior (29). Indeed, subjects with the *ALDH2*1/1* genotype drank alcohol more frequently than those with *ALDH2*1/2* and *2/2* (Table 3).

Table 1 Genotypic frequencies for *ALDH2*

Subjects	Number	*ALDH2* genotype			
		1/1	*1/2*	*2/2*	*p**
Male	1137	590 (51.9%)	449 (39.5%)	98 (8.6%)	
Female	1127	565 (50.1%)	461 (40.9%)	101 (9.0%)	0.71
Age					
40s	573	280 (48.9%)	241 (42.1%)	52 (9.1%)	
50s	561	285 (50.8%)	224 (39.9%)	52 (9.3%)	
60s	569	292 (51.3%)	222 (39.0%)	55 (9.7%)	
70s	561	298 (53.1%)	223 (39.8%)	40 (7.1%)	0.65
Total	2264	1155 (51.0%)	910 (40.2%)	199 (8.8%)	

*Comparison of genotype distributions by gender (Chi-square statistics)

Female	*ALDH2* genotype	
	1/1 and *1/2*	*2/2*
Age 40s and 50s	498 (89.25%)	60 (10.75%)
Age 60s and 70s	524 (92.91%)	40 (7.09%)

Chi-square statistics; $p = 0.031$
Fisher's exact test (left); $p = 0.020$

Table 2 Effects of *ALDH2* genotypes on serum lipid, lipid protein, and lipid peroxides

Gender	Male			Female		
ALDH2 genotypes Evaluations[b]	*1/1*	*1/2* & *2/2*	*p*[a]	*1/1*	*1/2* & *2/2*	*p*
TG	140.7 ± 3.9[c]	126.7 ± 4.1	0.014*	107.8 ± 2.6	110.5 ± 2.6	0.462
Total chol	212.4 ± 1.4	212.0 ± 1.4	0.838	226.8 ± 1.5	227.3 ± 1.5	0.819
HDL	58.84 ± 0.61	55.91 ± 0.63	0.001*	67.01 ± 0.64	65.06 ± 0.65	0.033*
LPO	3.118 ± 0.029	3.072 ± 0.030	0.267	2.815 ± 0.030	2.946 ± 0.030	0.002*
LDL	130.0 ± 1.3	133.7 ± 1.4	0.055	138.4 ± 1.5	140.5 ± 1.5	0.316
APO A1	145.5 ± 1.1	138.2 ± 1.2	< 0.001*	157.5 ± 1.1	153.8 ± 1.1	0.021*
APO A2	38.69 ± 0.24	37.11 ± 0.25	< 0.001*	38.13 ± 0.23	37.45 ± 0.23	0.042*
APO B	108.0 ± 1.0	108.7 ± 1.0	0.594	109.6 ± 1.1	110.8 ± 1.1	0.405
APO C2	4.778 ± 0.055	4.490 ± 0.057	< 0.001*	4.590 ± 0.053	4.510 ± 0.053	0.283
APO C3	11.47 ± 0.13	10.58 ± 0.14	< 0.001*	10.87 ± 0.11	10.76 ± 0.11	0.479
APO E	4.762 ± 0.053	4.568 ± 0.055	0.012*	5.010 ± 0.050	4.999 ± 0.051	0.886

*$p < 0.05$
[a]Comparison by *ALDH2* genotypes in each gender (Tukey-Kramer)
[b]TG; Triglyceride (mg/dl), Total chol, total cholesterol (mg/dl); HDL, high density lipoprotein cholesterol (mg/dl); LPO, lipid peroxide (nmol/ml); LDL, low-density lipoprotein cholesterol (mg/dl); Apo, apolipoprotein (mg/dl)
[c]Concentrations are means ± SDs

Table 3 *ALDH2* genotypes and alcohol-drinking behavior

Subjects	Number	*ALDH2* genotype	Drinking[a]		
			Yes	No	Total
Male	1137	*1/1*	475 (87.3%)	69 (8.6%)	544
		1/2	234 (56.6%)	179 (43.3%)	413
		2/2	4 (4.3%)	89 (95.7%)	93
Female	1127	*1/1*	187 (36.3%)	328 (63.7%)	515
		1/2	62 (14.8%)	356 (85.2%)	418
		2/2	4 (4.3%)	90 (95.7%)	94

[a]Alcohol drinking was defined as > 5 g alcohol per day

Thus, we excluded the effects of alcohol-drinking behavior from the association of the *ALDH2*-deficienct genotype with the evaluation.Data were analyzed with an adjustment for alcohol consumption by the least squares method in the general linear model (Table 4), and we found that the concentration of LPO in females differed significantly by *ALDH2* genotype. The concentration was higher in females carrying at least one *ALDH2*2* allele (2.922 nmol/ml) than those carrying *ALDH2*1/1* (2.781 nmol/ml; $p = 0.003$), indicating the possibility that oxidative stress increases in *ALDH2*-deficient individuals. To find why the significance was found only in the females, the general linear model was applied with both *ALDH2* genotype and alcohol consumption used as an independent variable. As a result, the concentration of LPO was significantly determined

Table 4 Effects of *ALDH2* genotypes on serum lipid, lipid protein, and lipid peroxides (LPO): Exclusion of effects of alcohol-drinking behavior

Gender	Male			Female		
ALDH2 genotypes Evaluations[b]	*1/1*	*1/2* & *2/2*	p[a]	*1/1*	*1/2* & *2/2*	p
TG	138.0 ± 4.3[c]	131.2 ± 4.5	0.288	109.7 ± 2.8	111.4 ± 2.8	0.679
Total chol	212.9 ± 1.5	211.4 ± 1.6	0.492	227.2 ± 1.5	226.0 ± 1.6	0.566
HDL	57.71 ± 0.65	56.69 ± 0.67	0.291	66.48 ± 0.66	64.68 ± 0.67	0.057
LPO	3.054 ± 0.031	3.075 ± 0.032	0.640	2.781 ± 0.033	2.922 ± 0.033	0.003*
LDL	131.8 ± 1.5	132.2 ± 1.5	0.835	139.3 ± 1.5	139.5 ± 1.6	0.921
APO A1	143.0 ± 1.2	140.9 ± 1.3	0.250	157.2 ± 1.2	154.3 ± 1.2	0.076
APO A2	38.12 ± 0.25	37.92 ± 0.26	0.586	38.20 ± 0.25	37.61 ± 0.25	0.101
APO B	108.6 ± 1.1	108.1 ± 1.1	0.750	109.9 ± 1.1	110.4 ± 1.1	0.774
APO C2	4.741 ± 0.060	4.596 ± 0.062	0.105	4.654 ± 0.056	4.547 ± 0.056	0.183
APO C3	11.22 ± 0.14	10.94 ± 0.15	0.173	10.98 ± 0.12	10.80 ± 0.12	0.270
APO E	4.727 ± 0.058	4.627 ± 0.060	0.253	5.031 ± 0.053	5.000 ± 0.054	0.693

*$p < 0.05$
[a]Comparison by *ALDH* genotypes in each gender (Tukey-Kramer)
[b]TG, Triglyceride (mg/dl); Total chol, total cholesterol (mg/dl); HDL, high density lipoprotein cholesterol (mg/dl); LPO, lipid peroxide (nmol/ml); LDL, low-density lipoprotein cholesterol (mg/dl); Apo, apolipoprotein (mg/dl)
[c]Concentrations are means ± SDs

Table 5 Determinants of lipid peroxide[a]

	Determinants[b]	p[c]
Male	*ALDH2*	0.640
	Alcohol	0.001
Female	*ALDH2*	0.003
	Alcohol	0.247

[a]Data were analyzed by the general linear model with both *ALDH2* genotype and alcohol consumption as an independent variable
[b]The *ALDH2* genotypes were categorized into two groups, *1/1* or *1/2 + 2/2*. "Alcohol" indicates alcohol consumption, which was assessed as a continuous (grams alcohol per day) variable
[c]*F*-test

by alcohol consumption ($p = 0.001$) in males while by ALDH2 genotype ($p = 0.003$) in females (Table 5). Thus, a concentration of LPO in males was influenced by alcohol-drinking behavior.

Discussion

ALDH2 plays a major role in the oxidation of acetaldehyde in vivo. Its low *K*m facilitates the rapid clearance of acetaldehyde following the administration of alcohol, and a deficiency of ALDH2 results in an ethanol-related sensitive response attributable to an elevated blood acetaldehyde level. Several reports have suggested that an increase in the acetaldehyde concentration is a risk for diabetes (Suzuki et al. 1996a, 1996b), cancer (Yokoyama et al. 1998), and hypertension (Itoh et al. 1997) and is associated with *ALDH2*2*. Thus, the risk for geriatric diseases, including myocardial infarction, has been mainly argued through an association with alcohol consumption.

Instead, this study has revealed that ALDH2 could contribute to the pathogenesis of various geriatric diseases by an alternative pathway. Since the LPO concentration in sera of females carrying *ALDH2*2* was higher, even after correcting for alcohol-drinking behavior, the increase of LPO in ALDH-deficient individuals would not be due to drinking. This suggests that ALDH2 might contribute to the elimination of not only acetaldehyde from ethanol but also aldehyde derivatives produced by oxidative stress. We found no significant difference in males in the concentration of serum LPO among *ALDH2* genotypes. However, an analysis in the general linear model with both the *ALDH2* genotype and alcohol consumption used as an independent variable indicates that a determinant of the concentration of LPO is alcohol consumption in males. This finding strongly suggesting that frequent alcohol-drinking behavior in males overrides the phenotype of the high serum LPO level. Alternatively, it cannot exclude the possibility that some hormonal regulation contributes to the peroxidation.

Our previous case-control study has revealed that ALDH2 deficiency is a risk factor for LOAD in a Japanese population, synergistically acting with APOE-ϵ4 (Kamino et al. 2000). Oxidative stress has been primarily implicated in mechanisms of AD brain degeneration (Markesbery and Carney 1999, Praticò and Delanty 2000). A mouse model of AD amyloidosis showed evidence of a systemic increase in urine, plasma, and brain LPO compared with wild-type mice (Praticò et al. 2001). The increase preceded the onset of amyloid deposition. Oxidative damage to the central nervous system predominantly manifests as LPO because of the high level of polyunsaturated fatty acids that are particularly susceptible to oxidation. Thus, the higher LPO concentration in sera of females carrying *ALDH2*2* might be reflected by the higher frequency of LOAD in females.

Mechanisms underlying the increase of LPO in ALDH2-deficient individuals should be further investigated. However, one possible explanation is as follows: The enhanced accumulation of toxic acetaldehyde or

aldehyde derivatives including 4-HNE in the ALDH2-deficient cells induces cell death, and cellular damage induced by the accumulated aldehyde derivatives further enhances oxidative stress in vivo. Several studies have revealed that 4-HNE, an aldehyde derivative of membrane lipid peroxidation, is a key mediator of neuronal apoptosis induced by oxidative stress and that a protein modification by 4-HNE increases in the AD brain (Montine et al. 1997, Sayre et al. 1997).

The metabolism of 4-HNE in hepatocytes has been reported to be dependent on three enzymatic pathways: oxidation with ALDH, reduction with alcohol dehydrogenase, and conjugation with glutathione (Hartley et al. 1995). Recently, we found that ALDH2 deficiency in PC12 cells increased cell death after treatment with cytotoxic 4-HNE (Ohsawa et al. 2003). Furthermore, after treatment with antimycin A, the ALDH2 deficiency resulted in an enhancement of both 4-HNE accumulation and cell death. These results strongly support our explanation described above.

Finally, our results suggest possible roles of ALDH2 deficiency in the increase in LPO, which is produced in response to oxidative stress. Even on excluding the effects of alcohol-drinking behavior from the association with the *ALDH2*-deficienct genotype using the correction, the concentration of LPO in females significantly differed by *ALDH2* genotype. However, the effect of frequent alcohol-drinking behavior in males seems to override the phenotype of the high serum LPO level. LPO and its derivative, 4-HNE, are involved in the pathogenesis of several geriatric diseases including AD, and ALDH2 may detoxify them. Thus, the metabolism of toxic aldehydes, including 4-HNE, could be a preventive and therapeutic target in geriatric diseases.

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